Mouse Paw Sensation and Response in Primal Somatosensory Cortex and Primal Motor Cortex

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Abstract

Animals respond to environmental changes through their perceived notion of the world. Thus, understanding how different regions of the brain communicate to effectively adapt to environmental factors is important. Here we placed head-fixed and genetically modified mice (GCaMP6s x CamKII-tTa) on a wheel with three different textures. They were imaged using a 2-photon laser and recorded for 40 minutes. The neural imaging of calcium fluorescence was processed with Suite2p. The behavioral videos were processed with DeepLabCut. The outputs of both systems were filtered, realigned, and analyzed using python to determine the connection between different sensory stimuli through paws and neural preference in the S1 and M1 cortex.

Introduction

Sensory Information strongly influences motor coordination. There have been many studies on rodent S1 and M1 cortex alignment and response, almost all of which has used whisker sensory information. Knowledge on sensory and motor cortex interaction is necessary to begin to understand many rehabilitation and neurodegenerative diseases [1]. This study will allow for an initial insight into paw sensory stimuli and the response it induces in the M1 and S1 cortex. The theory is that a closer relation can be drawn from rodent to human sensorimotor functionality through understanding how the S1 and M1 cortex in mice communicate when stimuli are interpreted through the mice's paws instead of its whiskers.

The main question addressed here is if there is any neural preference between different textured terrain. This is an initial question in understanding the paw sensation on mice and how this can influence their behavior. The mice used were genetically modified so that their neurons could be captured and recorded when firing using a 2-photon laser. The GCaMP6s is a calcium indicator that enables reliable detection of single action potentials [2]. Although this indicator is mainly used for visualizing neurons in the V1 cortex it can also be used for M1 and S1 since it sparsely indicates neurons in these areas of the brain. The mice were trained on the wheel over the course of a week, for 30 minute intervals. They are head-fixed, so training them prior to the experiment allowed for them to get familiar with the wheel and behave as normally as possible. Once they had been trained they were placed on a wheel with different textures and 40 minutes of neural and behavioral data were recorded and processed with Suite2p (S2P) and DeepLabCut (DLC), respectively.

Given neural data and limb placement over 40 minutes, the problem is to determine neuronal preference in both M1 and S1 in relation to different sensory stimuli. The quality of the neural preference assignment will be determined through the percentage of assignment (at least a portion of recorded neurons will need to have an assigned preference). It is expected that these responses remain significantly different across conditions and that smoother terrain will elicit a smaller neural response and therefore be the texture on which the mouse spends most of its time.

Methods

The dataset is available in Prof. Dadarlat's Lab at Purdue University. It includes Neural fluorescent recordings and limb tracking over 40 minutes of navigation. The neural data is initially processed with S2P. This outputs neural fluorescence and neural pill indexed over detected neurons. The behavioral data is initially processed with DLC. This outputs limb position (x and y coordinates, as well as pixel placement) over time. Neural data is sampled at ~7 frames/sec, while the behavioral data has a sampling frequency of 30Hz. To ensure alignment later, there is a rectangular signal that begins with the recording of the neural data but marks every behavioral frame. This is critical for the experiment as it allows for behavioral data to be directly linked to neural data.

The behavioral data used is only of the x-position of each limb (front and hind limb) and the x-position of the start and end of each texture (320-grit sandpaper and 80-grit sandpaper). These were labeled as follows: front limb (FL), hind limb (HL), 320-grit start (LG_start), 320- grit end (LG_end), 80-grit start (LG_start), 80-grit end (HG_end). A mouse was said to be on a specific sandpaper when its fl was behind the end of the paper, and it hl was in front of the start of the paper. If the mouse was on the smooth wheel its fl would be behind the start of the sandpaper, and hl would be in front of the end of the texture. When mice were found to be half-on a sandpaper this was also marked. There were a total of 5 different markings, 1–completely on 320-grit, 2-completely on 80-grit, 3-completely on the smooth wheel, 4-half-on the 320-grit, and 5-half-on the 80-grit.

Description of Signal Processing Step	Rationale	Inputs and outputs of the step
Linear Regression	Removing 70% of neuropil surrounding detected cells to better visualize and interpret correlation between behavior and neural response.	Inputs: Neural data (including Neural pill) Outputs: Cell fluorescence
High-pass Band Filter	Ensuring data is of 70% or greater accuracy. Limb placement from DLC is >95% correct and cell detection from S2P is >70%.	Input: Neural fluorescence + Behavioral data Output: Accurate cell detection and limb placement across video image.
Align Neural Data to First Behavioral Frame	In some cases the neural data started recording before the behavioral data, to ensure that all data matches up accordingly.	Input: Size of neural dataset + first frame behavioral data starts on (this is numbered according to S2P and output by DLC) Output: Neural data (excluding the initial datapoints that were mismatched with the behavioral dataset)
Match Neural Data to Behavioral Data	Since sampling frequencies are different for both datasets, this will ensure that all extra behavioral points are not taken into consideration and also	Input: Video frames (numbered from 1 to end) + total frames in behavioral dataset + empty behavioral matrix with total neural dataset size. Output: Readjusted behavioral frames (only accounting for every frame that aligns with neural frames by starting point).

Mask Behavioral Data According to New Frame Set	After determining what behavioral frames align with neural frames, we have to ensure the data is also aligned	Input: All data point for FL,HL,LG,HG (start and end) + behavioral frames that align with the neural data. Output: All limb and behavioral markers data for the predetermined frames.
Determine Mouse Placement for Every Frame	To determine what neural response was elicited by what stimuli we must determine the mouse position at every given behavioral and neural data point	Input: All limb and behavioral markers x-position + an empty location array (with the same size as total frames) Output: List of unique locations possible + amount of unique locations + array with every location in order of start to finish
Create Tuning Curves for Every Given Neuron	To analyze neural responses in relation to mouse position and wheel texture we take the average of responses of every neuron on each predetermined location.	Input: Array with mouse position assigned for every frame + Neural data + empty array for tuning curve responses Output: Tuning curve responses for every neuron over the five predetermined locations
Determine Max Neural Response for Every Neuron	The preferred stimuli of a neuron will elicit a maximum response, thus, to determine preferred textures to each neuron we must determine at what location its maximum response occurred	Input: tuning curve array Output: Maximum neural response of every neuron + texture that elicited said response

Results

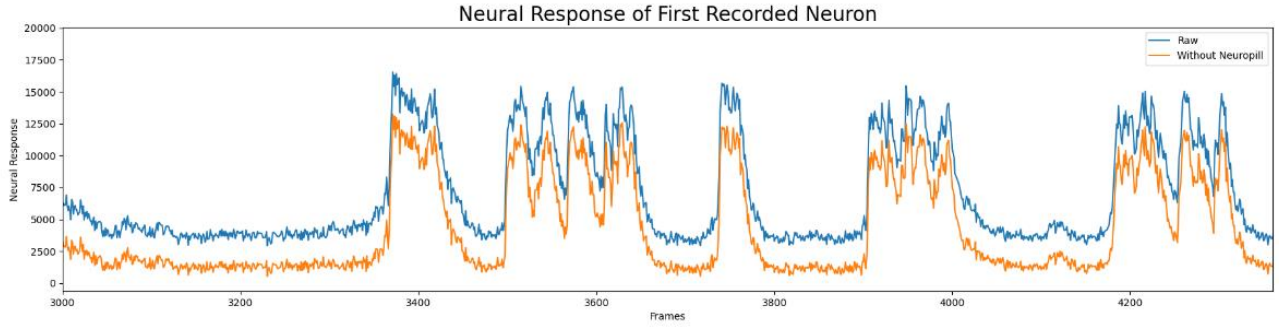

Figure 1: Neural Response of a Neuron. In blue we see the given neural response and in orange, the neural response without the neuropil fluorescence.

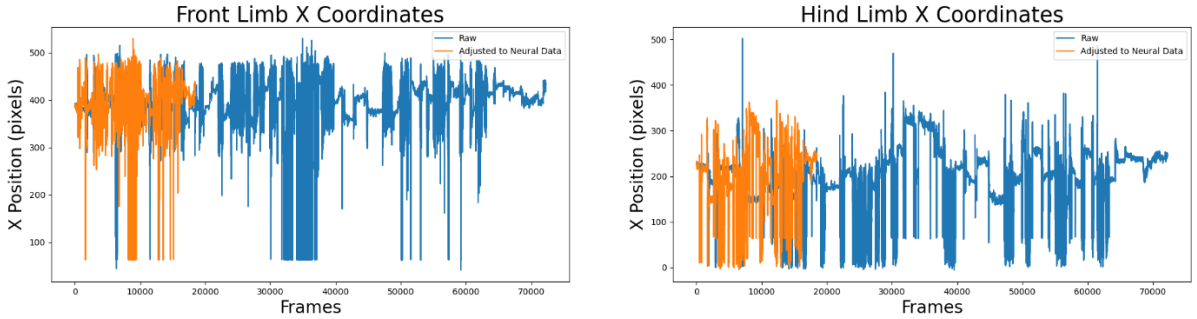

Figure 2: Front Limb and Hind Limb Frame Extraction. In blue the behavioral data over 72240 frames, in orange the behavioral data over 36132 frames

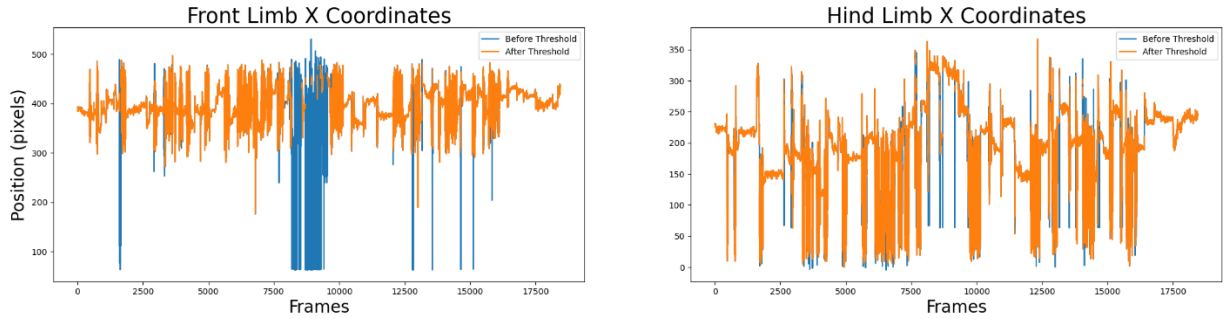

Figure 3: Front Limb and Hind Limb Data Filtering. In blue all the behavioral data for both limbs, in orange the behavioral data with 95% or more likelihood.

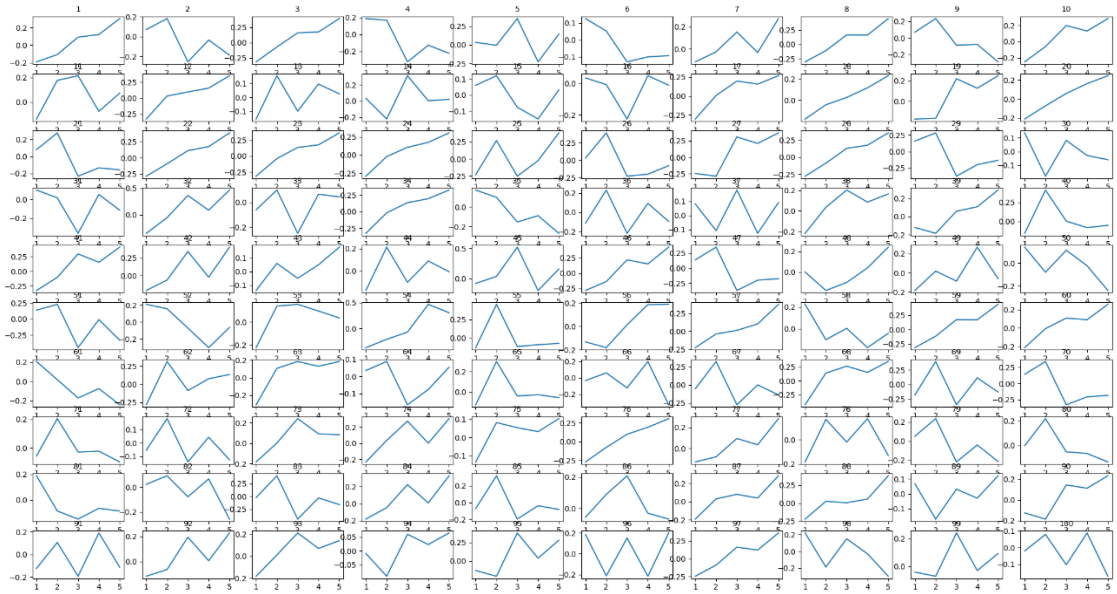

Figure 4: Tuning Curves for First 100 Neurons: Average Neural Response of Given Neuron X Mouse Location on Wheel. (1- 320-grit, 2- 80-grit, 3- Smooth Wheel, 4- Half-on 320-grit, 5- Half-on 80-grit)

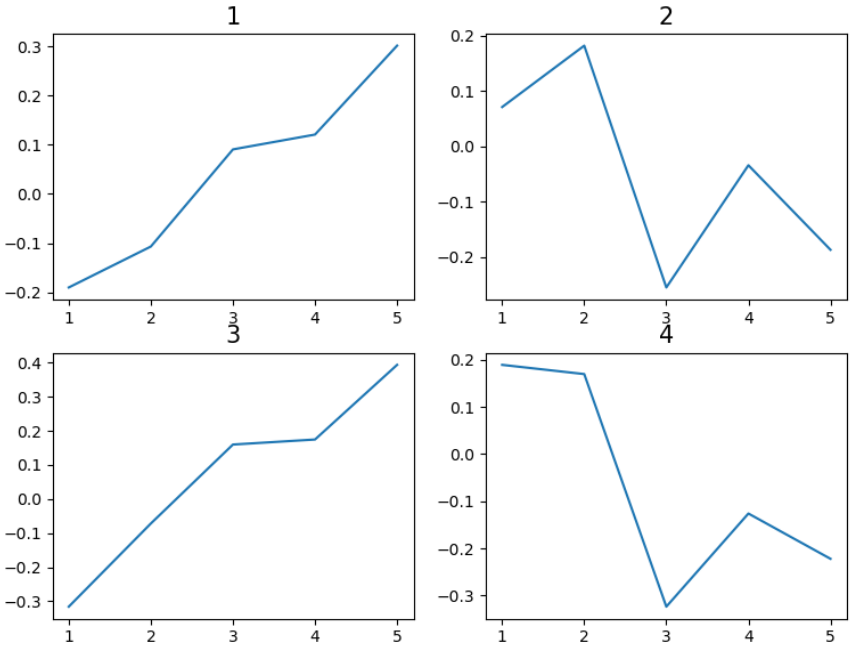

Figure 5: Tuning Curves for First 4 Neurons. Neuron 1 has a preference for 5; Neuron 2, a preference for 2; Neuron 3, a preference for 5; and Neuron 4 prefers 1. (1- 320-grit, 2- 80-grit, 3- Smooth Wheel, 4- Half-on 320-grit, 5- Half-on 80-grit)

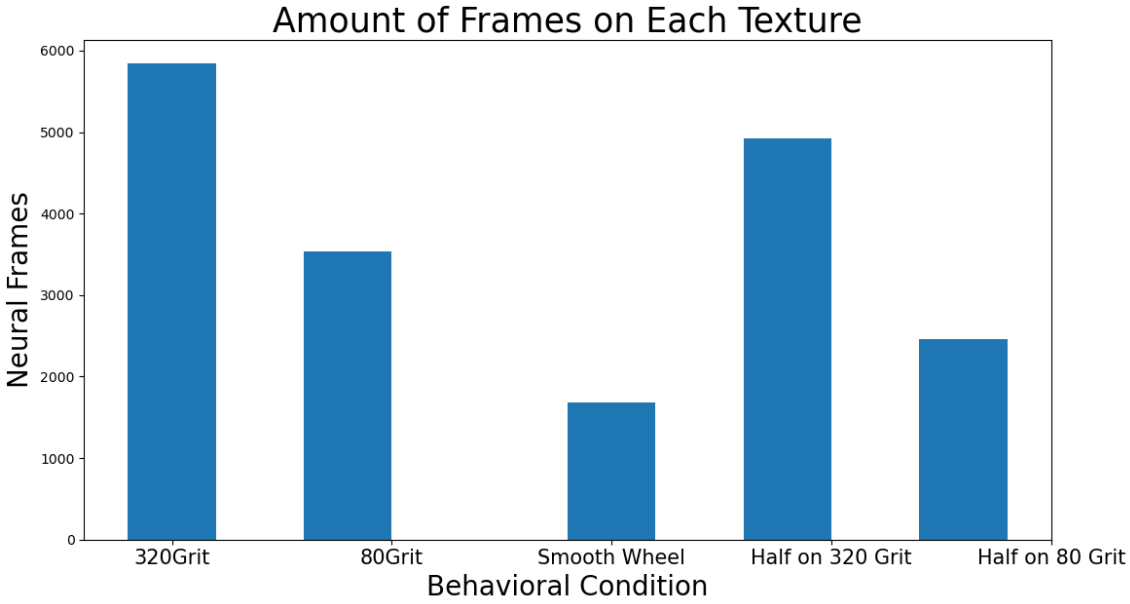

Figure 6: Total Number of Frames Spent on Each Texture.

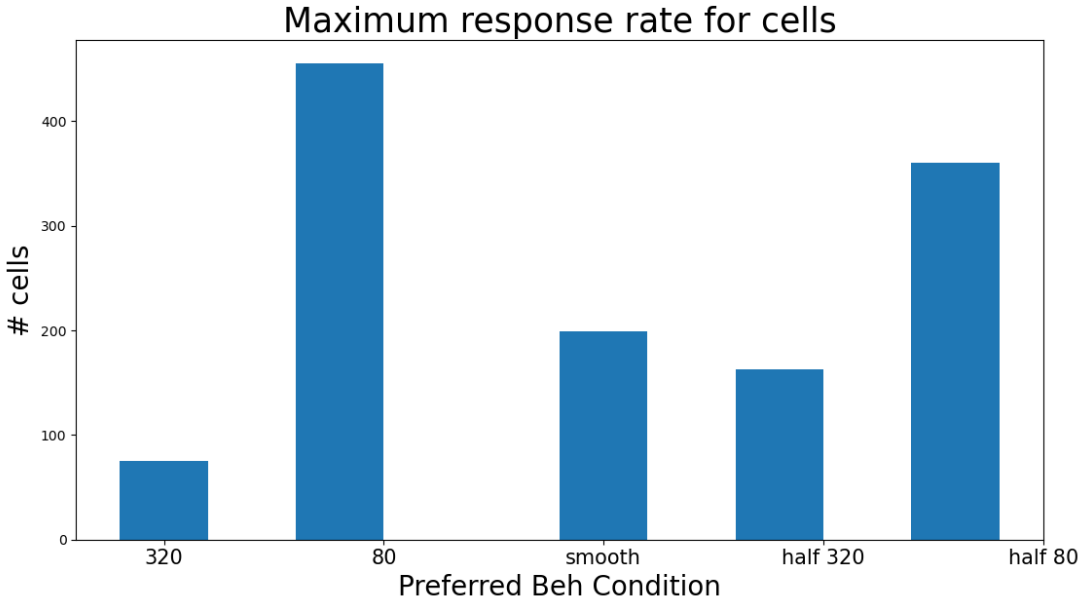

Figure 7: Total Number of Cells with a Preference for Each Texture.

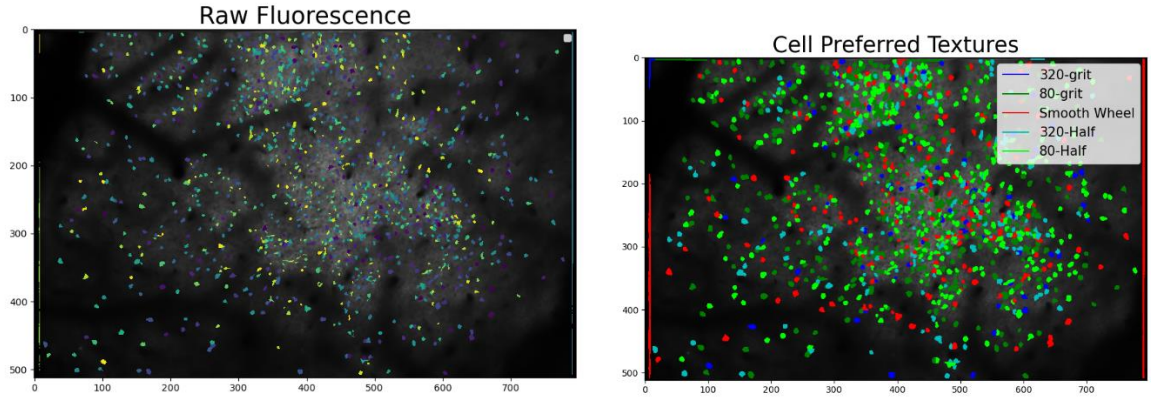

Figure 8 and 9: Cell Fluorescence over Mean Image of Brain, with Markers for Each Preferred Texture

Conclusion

After processing and analyzing 40 minutes of behavioral videos and neural data for 1 mouse, there is definitely a preferred texture for each recorded neuron. It can be seen in Figures 7 and 9, that the great majority of cells had hire firing rates when the mouse was on the 80-grit sandpaper, both half-on and completely on. In figure 6 it can be seen that the mouse spent a longer time on the 320-grit sandpaper. This is due to the low firing rates this paper elicited. This is in alignment with the initial assumption that a smoother surface would be preferred behaviorally due to smaller firing rates.

The reason as to why the smooth wheel was not the ultimately preferred surface over frames is unclear. Most likely due to the grates on the wheel which can be interpreted as grain but could also be due to the different material having a different temperature. More information would be needed to determine with certainty. In Figures 8 and 9, we can see that not a lot of cells are fluorescing in bot S1 and M1, this may have caused a skew in the data. Another issue that might have interfered with results is the false alarm rate that calcium markers cause, due to their slow decay rate. The over indexing of specific neurons and over representation of activity could be causing a difference in the overall results of this experiment. This is unlikely, since the sampling frequency of the neural data was so low.

References

[1] A. Fukui, H. Osaki, Y. Ueta, K. Kobayashi, Y. Muragaki, T. Kawamata, and M. Miyata, “Layer-specific sensory processing impairment in the primary somatosensory cortex after motor cortex infarction,” *Nature News*, 28-Feb-2020. [Online]. Available: https://www.nature.com/articles/s41598-020-60662-7. [Accessed: 16-Dec-2021].

[2] T.-W. Chen, T. J. Wardill, Y. Sun, S. R. Pulver, S. L. Renninger, A. Baohan, E. R. Schreiter, R. A. Kerr, M. B. Orger, V. Jayaraman, L. L. Looger, K. Svoboda, and D. S. Kim, “Ultrasensitive fluorescent proteins for imaging neuronal activity,” *Nature*, 18-Jul-2013. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3777791/. [Accessed: 16-Dec-2021].